A kind of polypeptide and application thereof with anti-aging and repair

Abstract

The invention discloses a kind of polypeptide and application thereof with anti-aging and repair.The present invention obtains a kind of novel polypeptide with anti-aging and efficient repair from marine organisms hydra, the polypeptide can promote fibroblastic growth, fibroblast is promoted to generate hyaluronic acid, therefore having, which improves skin quality collagen, is lost, reinforces moistening effect, efficiently repairs skin, highlighting whitening and crease-resistant and other effects, can be used for preparing anti-aging and repairs the drug or cosmetics of skin.

Classifications

A61K8/64 Proteins; Peptides; Derivatives or degradation products thereof

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CN110078793A
China

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Other languages: Chinese

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Current Assignee : Guangdong baioufei silk Cell Research Center Co.,Ltd.

Worldwide applications

2019 • CN

Application CN201910209385.5A events

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2020-10-16 • Publication of CN110078793B

Status • Active

2039-03-19 • Anticipated expiration

Info: Patent citations (4), Cited by (2), Legal events, Similar documents, Priority and Related Applications

External links: Espacenet, Global Dossier, Discuss

Claims (10)

Hide Dependent

1. a kind of polypeptide, which is characterized in that its amino acid sequence is as shown in SEQ ID NO.2.

2. a kind of encoding gene of polypeptide described in claim 1.

3. encoding gene according to claim 2, which is characterized in that its nucleotide sequence is as shown in SEQ ID NO.1.

4. a kind of prokaryotic expression carrier of the encoding gene containing polypeptide described in claim 2 or 3.

5. prokaryotic expression carrier according to claim 4, which is characterized in that the prokaryotic expression carrier is carrier pGEX-4T-1。

6. a kind of bacterium containing prokaryotic expression carrier as claimed in claim 4.

7. bacterium according to claim 6, which is characterized in that the bacterium is e. coli bl21 (DE3).

8. polypeptide described in claim 1 promotes fibroblastic growth in preparation or fibroblast is promoted to generate hyaluronic acid Preparation in application.

9. application of the polypeptide described in claim 1 in the drug or cosmetics of preparation anti-aging and reparation skin.

10. a kind of anti-aging and the drug or cosmetics for repairing skin, which is characterized in that including polypeptide described in claim 1 As active constituent.

Description

A kind of polypeptide and application thereof with anti-aging and repair

Technical field:

The invention belongs to medical cosmetic product fields, and in particular to a kind of polypeptide with anti-aging and repair and its Purposes.

Background technique:

The main body of skin is skin corium, is mainly made of fibroblast and matrix components.Fibroblast generates glue The glycosaminoglycans such as former albumen and hyaluronic acid form connective tissue, play an important role in skin.Fibroblast Function because The reduction of the other influences such as aging or ultraviolet light may result in the matrix components such as collagen, hyaluronic acid and reduce and be denaturalized. The oxidative stress such as ultraviolet light cause skin injury, keep its coarse, and cause other adverse reactions.Due to matrix components reduction and Oxidative stress accelerates skin that aging occurs, and wrinkle, spot, appearance dimness, texture thus occurs and loses smooth, elastic reduction etc. Aging sign is presented.

Collagen and hyaluronic acid have been main trends instantly as the direction of prevention skin aging.It analyses scientifically transparent Matter acid is a kind of high molecular polymer, and the way that hyaluronan extraction object is applied to skin is directly detrimental to skin and is absorbed It utilizes.Hydra body is mainly made of stem cell, while the content of hyaluronic acid and collagen is higher, can maintain hydra The ability that stem cell is constantly divided.

Summary of the invention:

The object of the present invention is to provide a kind of polypeptide and application thereof with anti-aging and repair.

It is of the invention the study found that extracting a kind of biological polypeptide (its amino acid sequence such as SEQ ID NO.2 institute from hydra Show) fibroblastic growth can be promoted from cell level, stimulation fibroblast generates hyaluronic acid.

Therefore, the first purpose of the invention is to provide a kind of polypeptides, and amino acid sequence is as shown in SEQ ID NO.2.

A second object of the present invention is to provide the encoding gene of the polypeptide, nucleotide sequence such as SEQ ID Shown in NO.1.

Third object of the present invention is to provide a kind of prokaryotic expression carriers of encoding gene containing the polypeptide.

The prokaryotic expression carrier is preferably vector pGEX -4T-1.

Fourth object of the present invention is to provide a kind of bacterium containing the prokaryotic expression carrier.

The bacterium is preferably e. coli bl21 (DE3).

Fifth object of the present invention is to provide the polypeptides in the preparation that preparation promotes fibroblastic growth Using.

Sixth object of the present invention is to provide the polypeptides to promote fibroblast to generate hyaluronic acid in preparation Application in preparation.

7th purpose of the invention is to provide the polypeptide in the drug or makeup of preparation anti-aging and reparation skin Application in product.

8th purpose of the invention is to provide the drug or cosmetics of a kind of anti-aging and reparation skin, including described Polypeptide is as active constituent.

The cosmetics can be prepared into various dosage forms such as freeze-dried powder, Essence, lotion, creme etc..

The present invention obtains the polypeptide of a kind of novel anti-aging and efficient repair from marine organisms hydra, the polypeptide energy Enough promoting fibroblastic growth, stimulation fibroblast generates hyaluronic acid, therefore there is improvement skin quality collagen to be lost, Reinforce moistening effect, efficiently repair skin, highlight whitening and crease-resistant and other effects, can be used for preparing anti-aging and repair skin Drug or cosmetics.

Detailed description of the invention:

Fig. 1 is influence of the polypeptide (its amino acid sequence is as shown in SEQ ID NO.2) to fibroblastic growth.

Fig. 2 is the shadow that polypeptide (its amino acid sequence is as shown in SEQ ID NO.2) generates hyaluronic acid to fibroblast It rings.

Specific embodiment

The following examples are further illustrations of the invention, rather than limiting the invention.

Embodiment 1:

1, RNA is extracted

The extraction of total serum IgE is extracted using Trizol (Invitorgen), the specific steps are as follows:

(1) it takes 50mg clear water Xi Hydra viridis global tissue in the mortar of Liquid nitrogen precooler, tissue is clayed into power Later, it is transferred in the centrifuge tube equipped with 1mL Trizol, piping and druming mixes;

(2) 200 μ L chloroforms are added, acutely shakes 40s, is placed at room temperature for 5min;

(3) 4 DEG C, 12,000 × g is centrifuged 10min, draws supernatant and is transferred to new pipe;

(4) isopropanol of 0.5mL is added, mixes, -80 DEG C of precipitates overnights;

(5) 4 DEG C, 12,000 × g is centrifuged 10min, abandons supernatant;

(6) 75% ethyl alcohol wash are precipitated twice;

(7) supernatant is abandoned, 100 μ L DEPC water are added and dissolve RNA.

2, cDNA library constructs

CDNA library building uses SMART cDNA library construction Kit (Clontech), tool Steps are as follows for body:

(1) RNA for utilizing step 1 synthesizes first chain of cDNA using MMLV;

(2) LD PCR amplification cDNA is used;

(3) protease K digesting and cDNA product is purified;

(4) SfiI digests, and carries out primer size separation using Chroma Spin400;

(5) cDNA to λ TripEx2 carrier is connected, after λ bacteriophage is packed, is converted into host cell;

(6) cDNA library titre is detected.

3, Prokaryotic expression vector construction

(1) design a pair covers the primer (being shown in Table 1) of hydra polypeptide sequence, the both ends of primer be separately added into BamH-I and The site Xho-I and protection base;

1 primer sequence of table

(2) using clear water Xi cDNA as template, PCR amplification is carried out using the primer of step (1);

(3) PCR product and pGEX-4T-1 carrier use BamH-I and Xho-I to carry out double digestion, and recovery purifying enzyme respectively Cut product;

(4) PCR product and pGEX-4T-1 carrier after connecting digestion;

(5) connection product being converted into bacillus coli DH 5 alpha, picking positive colony, sequence verification carrier is correct, Sequencing result shows that the sequence as shown in SEQ ID NO.1 is properly inserted into pGEX-4T-1 carrier, thus obtains propolypeptide Nuclear expression carrier carries out follow-up test.

4, the prokaryotic expression of recombinant protein and purifying

(1) the polypeptide prokaryotic expression carrier in the bacillus coli DH 5 alpha of extraction step 3, conversion to e. coli bl21 (DE3);

(2) it selects monoclonal and cultivates to OD=0.6, be added IPTG to final concentration of 0.1mM, 22 DEG C, the item of 180rpm Inducing expression under part;

(3) bacterium solution is collected after 4h, 4 DEG C, 12,000 × g is centrifuged 10min;

(4) Lysozyme to final concentration 2mg/mL, then ultrasonication is added;

(5) Glutathione Sepharose 4B (GE Healthcare) purification of recombinant proteins is used, in 12%SDS- PAGE glue analyzes purified product, the polypeptide purified (its amino acid sequence is as shown in SEQ ID NO.2).

5, measurement polypeptide promotes the activity of fibroblastic growth

(1) taking human dermal fibroblasts system BJ cell inoculation, (100 holes μ L/, cell inoculation amount are 1 in 96 orifice plates $\times 10^4$A/hole), in 37 DEG C, 5%CO_2(culture medium is not add the L-15 of growth stimulator containing cow's serum for bed board culture in incubator Basal medium).

(2) the purified 100 μ L of polypeptide solution of step 4 is added into culture medium after cultivating 6h, and makes polypeptide final concentration point Wei not be 5,10,20,30 μM (polypeptide group), taking one group of 3 repetition to add isometric PBS to do control is concentration 0 (PBS group), also Negative control is not added cell list and adds culture medium.

(3) cell cultivates 48h in the incubator, and 10 μ L MTT solution (5mg/mL) are added in every hole, continues to train in incubator 3h is supported, inhales and abandons culture medium, 100 μ L DMSO of every hole addition, which shake to crystallization, is completely dissolved (MTT cell viability detection kit).

(4) absorbance of microplate reader detection 450nm and 625nm, calculates fibroblastic growth rate.

(5) it is presented as the result is shown in 5~30 μM of concentration of range polypeptide and promotes the trend of fibroblastic growth, and polypeptide The cell growth rate of group (final concentration of 10 μM of polypeptide) is approximately about 2.6 times (see Fig. 1) of PBS group.

6, the effect that measurement polypeptide promotes hyaluronic acid to generate

(1) amount for the hyaluronic acid that ELISA method measurement fibroblast generates.

(2) by human dermal fibroblasts system BJ cell inoculation, (200 holes μ L/, cell inoculation amount are 1 in 48 orifice plates $\times 10^5$A/hole, culture medium is using the L-15 basal medium containing cow's serum but without growth stimulator), in 37 DEG C, 5%CO_2In incubator It is incubated overnight.

(3) after for 24 hours, toward every group of 20 μ L of addition polypeptide solution, make polypeptide final concentration be respectively 0,100,200,300,400, 500 μM, incubator culture 48h.

(4) supernatant is collected by centrifugation in every group of cell culture.

(5) content of hyaluronic acid in hyaluronic acid assay kit ELISA method measurement supernatant is utilized.

(6) as the result is shown polypeptide within the scope of a certain concentration, hyaluronic acid contents present ascendant trend, after tend towards stability (see Fig. 2).

Sequence table

<110>Guangdong Tai Nuo stem cell biological Science and Technology Ltd.

<120>a kind of polypeptide and application thereof with anti-aging and repair

<160> 2

<170> SIPOSequenceListing 1.0

<210> 1

<211> 60

<212> DNA

<213>clear water Xi (Hydra viridis)

<400> 1

gaatatttct ccgaattctc caaagaattc gtgtccgaag aatcctcctt ccttgaattc 60

<210> 2

<211> 20

<212> PRT

<213>clear water Xi (Hydra viridis)

<400> 2

Leu Ile Lys Arg Leu Lys Arg Phe Leu Lys His Arg Leu Leu Arg Arg

1 5 10 15

Lys Glu Leu Lys

20

Patent Citations (4)

Publication number	Priority date	Publication date	Assignee	Title
DE19808258A1 *	1997-02-28	1998-09-03	Evotec Biosystems Gmbh	Hydra head activator binding protein
WO2010063250A1 *	2008-12-06	2010-06-10	Christian-Albrechts-Universität Zu Kiel	Antimicrobial peptides made of hydra
CN108586575A *	2018-04-11	2018-09-28	福建省中科生物股份有限公司	A kind of application of polypeptide and its skin repair function
CN108794592A *	2018-06-29	2018-11-13	浙江辉肽生命健康科技有限公司	A kind of biologically active polypeptide NAGVLQDIRFKQ and its preparation method and application
Family To Family Citations				

* Cited by examiner, † Cited by third party

Cited By (2)

Publication number	Priority date	Publication date	Assignee	Title

Publication number	Priority date	Publication date	Assignee	Title
CN113201057A *	2021-03-16	2021-08-03	广东佰欧斐丝细胞科研中心有限公司	Deep-sea top clam antibacterial protein and application thereof
CN113876928A *	2021-10-22	2022-01-04	北京远胜达生物科技发展有限公司	Preparation of fibroblast outer vesicle and application of fibroblast outer vesicle in beauty treatment and medicines
Family To Family Citations				

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
FI3778885T3	2023-05-23	COMPOSITIONS AND METHODS OF ENGINEERED HUMAN ARGINASES FOR TREATMENT OF CANCER
CN107619437B	2019-08-13	A kind of skin ultrastructure peptide WHPP-OA1 and its method of purification and application
CN101698682A	2010-04-28	Double-functional fusion protein based on antibacterial peptide, preparation method and applicaitoin thereof
NZ627941A	2015-01-30	Hatching fluid enzymes and uses thereof
CN104402975A	2015-03-11	Anti-aging short peptide and preparation method thereof
CN102757974B	2014-07-23	Novel preparation method for recombinant human epidermal growth factor
CN110078793A	2019-08-02	A kind of polypeptide and application thereof with anti-aging and repair
KR20140140396A	2014-12-09	Composition for improving skin wrinkles and skin whitening
CN112876569B	2022-05-10	rhTSG6-FN III1-C fusion protein, application thereof in skin care composition and preparation method thereof
CN107011430A	2017-08-04	A kind of growth and differentiation factor 11 of the truncation with biological activity and preparation method thereof
CN105131083A	2015-12-09	Flat almond peptides capable of inhibiting activity of angiotensin converting enzyme (ACE) and preparation method thereof
CN114957394B	2023-06-09	Polypeptide PM-7 for promoting skin repair and application thereof
CN110585445A	2019-12-20	Application of mushroom serine protease inhibitor in preparing sobering-up and liver-protecting medicine and medicine for preventing and treating alcoholic liver injury
CN114196640B	2024-07-09	L-carnosine synthetase ATPGD derived from novel shellfish and application thereof

Publication	Publication Date	Title
CN101580846A	2009-11-18	Human cytoglobin for preventing and curing cirrhosis and preparation method thereof
CN111286511B	2022-11-25	A method and application of producing human epidermal growth factor and Ganoderma lucidum immunoregulatory protein
CN114702549A	2022-07-05	Active hexapeptide with antioxidation effect and application thereof
CN114699506A	2022-07-05	Use of recombinant calreticulin for growing hair, protecting hair or preventing alopecia and related products
HUP0001057A2	2000-08-28	Bovine lactation associated immunotropic protein (cd14), encoding gene and application in b cell activation
TWI846476B	2024-06-21	Recombinant human type iii collagen and nucleotide sequence, preparation method, and use thereof
CN104829691A	2015-08-12	Wrinkle-eliminating oligopeptide and preparation method thereof
RU2601126C2	2016-10-27	Method of producing biologically active peptides
CN100334114C	2007-08-29	Novel fusion protein production and uses
FR2900407B1	2008-07-25	NOVEL POLYPEPTIDES INDUCING DENDRITIC CELLS AND MEDICAMENTS AND PHARMACEUTICAL COMPOSITIONS CONTAINING SUCH POLYPEPTIDES
CN100383183C	2008-04-23	Algae enzyme hydrolyzate and production method thereof

Priority And Related Applications

Priority Applications (1)

Application	Priority date	Filing date	Title
CN201910209385.5A	2019-03-19	2019-03-19	Polypeptide with anti-aging and repairing functions and application thereof

Applications Claiming Priority (1)

Application	Filing date	Title
CN201910209385.5A	2019-03-19	Polypeptide with anti-aging and repairing functions and application thereof

Legal Events

Date	Code	Title	Description
2019-08-02	PB01	Publication	

Date	Code	Title	Description
2019-08-02	PB01	Publication	
2019-08-27	SE01	Entry into force of request for substantive examination	
2019-08-27	SE01	Entry into force of request for substantive examination	
2020-10-16	GR01	Patent grant	
2020-10-16	GR01	Patent grant	
2022-01-28	TR01	Transfer of patent right	**Effective date of registration**: 20220117 **Address after**: 510700 Room 203, building 3, No. 9, Xiangshan Road, Huangpu District, Guangzhou City, Guangdong Province **Patentee after**: Guangdong baioufei silk Cell Research Center Co.,Ltd. **Address before**: 510000 210, No. 110, Zhucun East Ring Road, Tianhe District, Guangzhou City, Guangdong Province **Patentee before**: Guangdong Peptide Normal Stem Cell Biotechnology Co.,Ltd.
2022-01-28	TR01	Transfer of patent right	

Concepts

machine-extracted

Download Filter table

Name				Image	Sections	Count	Query match
polypeptide	title,claims,description	37	0.000				
processed proteins & peptides	title,claims,description	37	0.000				
processed proteins & peptides	title,claims,description	37	0.000				
anti-aging effect	title,claims,description	11	0.000				
repair process	title,description	8	0.000				

Name				Image	Sections	Count	Query match
(2S,3S,4S,5R,6R)-6-[(2S,3R,4R,5S,6R)-3-Acetamido-2-[(2S,3S,4R,5R,6R)-6-[(2R,3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6-(hydroxymethyl)oxan-4-yl]oxy-2-carboxy-4,5-dihydroxyoxan-3-yl]oxy-5-hydroxy-6-(hydroxymethyl)oxan-4-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid	claims,description	14	0.000				
hyaluronan	claims,description	14	0.000				
hyaluronic acid	claims,description	13	0.000				
fibroblast	claims,description	11	0.000				
prokaryotic expression	claims,description	10	0.000				
fibroblastic effect	claims,description	8	0.000				
growth	claims,description	8	0.000				
preparation method	claims,description	8	0.000				
Bacteria	claims,description	6	0.000				
cosmetic	claims,description	6	0.000				
Escherichia coli	claims,description	5	0.000				
drug	claims,description	5	0.000				
drug	claims,description	5	0.000				
proteins and genes	claims,description	5	0.000				
constituent	claims,description	2	0.000				
nucleotide	claims,description	2	0.000				
nucleotide group	claims,description	2	0.000				
alpha amino acid group	claims	1	0.000				

Show all concepts from the description section